Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/010801

International filing date: 30 March 2005 (30.03.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/558,047
Filing date: 31 March 2004 (31.03.2004)

Date of receipt at the International Bureau: 06 May 2005 (06.05.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
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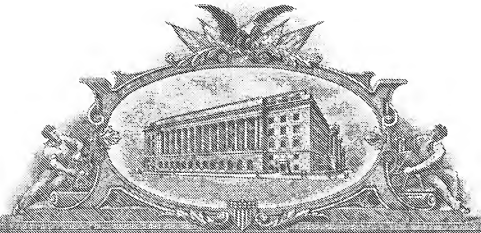

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APPLICATION NUMBER: ***60/558,047***
FILING DATE: ***March 31, 2004***
RELATED PCT APPLICATION NUMBER: ***PCT/US05/10801***

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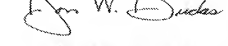

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15992 U.S.PTO 033104

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70377 U.S.PTO 60/556047 033104

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a **PROVISIONAL APPLICATION FOR PATENT** under 37 CFR 1.53 (c).

Express Mail Label No. EV326919933US

INVENTOR(S)

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☒ *Additional inventors are being named on the* 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

MACROMOLECULAR THERAPY FOR THE TREATMENT OF ARTHRITIS AND OTHER INFLAMMATORY DISEASES

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 39 ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* 7 ☒ Other (specify) Return Receipt Postcard

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.

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FILING FEE AMOUNT ($) 80.00

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☒ Yes, the name of the U.S. Government agency and the Government contract number are: EB00251.

Respectfully submitted,

SIGNATURE

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Date 3/31/04

REGISTRATION NO. *(if appropriate)* 33,041

Docket Number: 0274-6392US

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Docket Number	0274-6392US

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PATENT
Attorney Docket 0274-6392US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: EV326919933US

Date of Deposit with USPS: March 31, 2004

Person making Deposit: Christopher Haughton

PROVISIONAL APPLICATION

for

MACROMOLECULAR DELIVERY SYSTEMS FOR NON-INVASIVE IMAGING, EVALUATION AND TREATMENT OF ARTHRITIS AND OTHER INFLAMMATORY DISEASES

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MACROMOLECULAR DELIVERY SYSTEMS FOR NON-INVASIVE IMAGING, EVALUATION AND TREATMENT OF ARTHRITIS AND OTHER INFLAMMATORY DISEASES

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0001] Work described herein was supported in part by National Institute of Health Grant No. EB00251. The United States Government may have certain rights in the invention.

TECHNICAL FIELD

[0002] This invention relates to biotechnology, more particularly, to water-soluble polymeric delivery systems for non-invasive imaging, evaluation and treatment of arthritis and other inflammatory diseases.

BACKGROUND

[0003] Rheumatoid arthritis (RA) is the most common inflammatory arthritis, affecting about 1 percent of the general population worldwide. In United States, about 4.5 % of people over the age of 55 people have been affected (1, 2).

[0004] As a symmetric disease, RA usually involves the same joints on both sides of the body. Angiogenesis and microvascular lesions are common features of RA inflammation, which leads to abnormal serum protein infiltration into the synovia (3-5). Clearance of synovial fluid and its constituents was reported to be increased in inflamed joints as a result of increased lymphatic drainage (4), though damaged or depleted lymphatics have been observed in the synovium of RA patients as well (6, 7).

[0005] Although the exact cause of rheumatoid arthritis is unknown, many medications have been developed to relieve its symptoms and slow or halt its progression. Most commonly used medications rest on two principal approaches: symptomatic treatment with non-steroidal anti-inflammmatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs) (3).

[0006] Considerable effort has been made to identify and develop new therapeutic strategies for the treatment of RA. Novel RA medications, including, but not limited to, cycloxygenase-2 (COX-2) specific inhibitor (a NSAID) (8), tumor necrosis factor (TNF) blockers and interleukin-1 receptor antagonists (IL-1Ra) (DMARDs), have

been used for clinical applications (3). However, little attention has been paid to the pharmaceutical improvement of the available RA drugs to make them more efficient. In fact, many of the available treatments have various side effects, which limit their clinical application. Well-known side effects of NSAIDs include indigestion, stomach bleeding, liver and kidney damage, ringing in ears (tinnitus), fluid retention, and high blood pressure (9). Well known side effects of glucocorticoids include bruising, thinning of bones, cataracts, weight gain, redistribution of fat, diabetes and high blood pressure (10). Some DMARDs are immunosuppressants and their application can potentially lead to serious side effects, such as increased susceptibility to infection (3). Even with the newly developed COX-2 specific inhibitors, there are concerns about their side effects on kidney and bone (9).

[0007] With oral or systemic administration of these drugs, some side effects arise from their profound physiological activity in many tissues other than the inflamed joints. Therefore, the clinical dosing range of these therapeutics is kept relatively low to avoid the side effects, which would also reduce the efficacy of the drugs on the RA joints. Therapeutic delivery systems, which could specifically deliver anti-arthritis drugs to the inflamed joints of RA patients may avoid many of the side effects expressed in other tissues while achieving much greater clinical therapeutic efficacy.

[0008] The application of water-soluble polymers as a drug carrier for effective delivery of the drug to the desired sites (macromolecular therapy) has been extensively studied for the past two decades in the treatment of solid tumors (11). Because of the "leaky" vasculature and poorly developed lymphatic system, extravasated macromolecules can be efficiently accumulated in the solid tumor. This phenomenon is termed tumor-selective "enhanced permeability and retention" (EPR) and has been used successfully to target anti-cancer drugs to solid tumors (12).

[0009] Studies using micro-particular carriers, such as liposomes for the delivery of anti-arthritic agents to the RA joint have shown some promising results in an animal model of arthritis (13). However, because of the existence of lymphatic drainage, different retention effects on macromolecules may be expected in RA joints compared to a solid tumor.

SUMMARY OF THE INVENTION

[0010] The invention relates to water-soluble polymeric delivery systems. In one embodiment, the delivery system is used for delivery of anti-inflammatory drugs to the diseased sites for the treatment of arthritis and other inflammatory diseases. In another embodiment, the delivery system is used for delivery of imaging agents to the diseased sites for non-invasive imaging and evaluation of the diseased sites of arthritis and other inflammatory diseases.

[0011] In an exemplary embodiment, the invention provides a water-soluble polymeric delivery system for delivery of imaging agents, which are useful for non-invasive imaging and evaluation of arthritic joints and other inflammatory diseased organs or tissues. The imaging agents may be selected from any of the known compounds, for example, compounds useful for MRI, PET, CT or γ-scintigraphy imaging, etc. and mixtures thereof, such agents are well-known to those of skill in the art.

[0012] In another exemplary embodiment, the invention provides water-soluble delivery systems for the delivery of anti-inflammatory therapeutic agents selected from the group consisting of proteins, peptides, NSAIDs, glucocorticoids, methotrexate, sulfasalazine, chloriquine, gold, gold salt, copper, copper salt, penicillamine, D-penicillamine, cyclosporine, etc. and mixtures thereof, such drugs are well-known to those of skill in the art (37, 38).

[0013] In another exemplary embodiment, the inflammatory disease is rheumatoid arthritis, osteoarthritis, TMJ, inflamed nerve root, Crohn's disease, Chronic obstructive pulmonary disease, psoriasis diseases, asthma, colitis, multiple sclerosis, lupus, erythematosus, atherosclerosis and/or the like.

[0014] In another exemplary embodiment, the invention relates to drug delivery systems comprising a water-soluble polymer backbone, optionally, a targeting moiety or moieties, and a therapeutic agent or agents. The linkage (or linkages) between the targeting moiety (or moieties) and the polymer backbone is non-degradable or degradable under physiological conditions. The linkage (or linkages) between the therapeutic agent (or agents) and the polymer backbone is non-degradable or degradable under physiological conditions.

[0015] In another exemplary embodiment, the invention relates to delivery systems for imaging agents comprising a water-soluble polymer backbone, optionally, a targeting moiety or moieties, and an imaging agent or agents. The linkage (or linkages) between the targeting moiety (or moieties) and the polymer backbone is non-degradable or degradable under physiological conditions. The linkage (or linkages) between the therapeutic agent (or agents) and the polymer backbone is non-degradable or degradable under physiological conditions.

[0016] In yet another exemplary embodiment, the invention provides a method of manufacturing a pharmaceutical composition and/or medicament.

[0017] As will be apparent to a person of ordinary skill in the art based on the invention described herein, the invention provides the advantage of incorporating multiple therapeutic agents, targeting moieties, bio-assays labels, spacers and/or imaging agents, which may include a plurality of different chemical species from one or more of these groups. Therefore, in yet another exemplary embodiment the therapeutic agents, targeting moieties, bio-assays labels, spacers and/or imaging agents may consists of any number or combination of different species, having the same or different effects.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIG. 1. illustrates the chemical structure of a exemplary polymeric delivery system for MRI contrast agent (DOTA-Gd^{3+}), abbreviated as P-DOTA-Gd^{3+}, for the imaging of arthritic joints and evaluation of the severity of the disease.

[0019] FIG. 2. shows the histology of the ankle and knee joints from the same AIA rats imaged by MRI. FIG. 2A is a low power micrograph of the ankle and foot bones. Extensive swelling and inflammation is evident in the soft tissues (*) surrounding the foot bones. T = tibia. FIG. 2B shows the tarsal joint illustrating inflamed synovium (synovitis), extensive inflammatory infiltration (*) and cartilage and bone destruction. B = bone, A = articular cartilages. FIG. 2C is a higher power micrograph of the inflamed synovium. A = articular cartilage. FIG. 2D shows extensive bony destruction with inflammatory infiltration (*) in a tarsal (ankle) bone. Bone surfaces are lining with large active osteoclasts (arrows). FIG. 2E illustrates several blood vessels in an inflamed region of the ankle joint illustrating the inflammatory reaction around the vessels. The endothelial lining is thickened and vacuolated (arrows). FIG. 2F is a low power

micrograph of the knee joint from this same animal. There joint is quite normal in appearance except for a small pocket of inflammation on the posterial aspect of the joint (arrow). This same area was contrasted when observed by MRI. T = tibia; F = femur.

[0020] FIG. 3 shows the MR images of the animals taken at different time points. The acquired images were post processed using the maximum intensity projection (MIP) algorithm. FIG. 3A shows AIA rat baseline; FIG. 3B shows AIA rat, 5 min post injection of P-DOTA-Gd^{3+}; FIG. 3C shows AIA rat, 1 h post injection of P-DOTA-Gd^{3+}; FIG. 3D shows AIA rat, 2 h post injection of P-DOTA-Gd^{3+}; FIG. 3E shows AIA rat, 3 h post injection of P-DOTA-Gd^{3+}; FIG. 3F shows AIA rat, 8 h post injection of P-DOTA-Gd^{3+}; FIG. 3G shows AIA rat, 32 h post injection of P-DOTA-Gd^{3+}; FIG. 3H shows AIA rat, 48 h post injection of P-DOTA-Gd^{3+}; FIG. 3I. Healthy rat, baseline; FIG. 3J shows healthy rat, 5 min post injection of P-DOTA-Gd^{3+}; FIG. 3K. Healthy rat, 1 h post injection of P-DOTA-Gd^{3+}; FIG. 3L shows healthy rat, 2 h post injection of P-DOTA-Gd^{3+}; FIG. 3M. Healthy rat, 8 h post injection of P-DOTA-Gd^{3+}; FIG. 3N shows healthy rat, 48 h post injection of P-DOTA-Gd^{3+}; FIG. 3O shows AIA rat, 5 min post injection of OMNISCAN; FIG. 3P shows AIA rat, 2 h post injection of OMNISCAN; FIG. 3Q shows AIA rat, 8 h post injection of OMNISCAN; FIG. 3R shows AIA rat, 32 h post injection of OMNISCAN; FIG. 3S shows AIA rat, 48 h post injection of OMNISCAN; FIG. 3T shows healthy rat, 5 min post injection of OMNISCAN; FIG. 3U shows healthy rat, 1 h post injection of OMNISCAN; FIG. 3V shows healthy rat, 2 h post injection of OMNISCAN; FIG. 3W shows healthy rat, 8 h post injection of OMNISCAN; FIG. 3X shows healthy rat, 48 h post injection of OMNISCAN.

[0021] FIG. 4 illustrates single-plane MR imaging of AIA rats injected with P-DOTA-Gd^{3+}. FIG. 4A shows baseline MR image of AIA rat; FIG. 4B shows an MR image of AIA rat's left ankle and paw, 2 h post injection; FIG. 4C shows an MR image of AIA rat's left ankle and paw, 8 h post injection; FIG. 4D shows an MR image of AIA rat's left knee joint, 8 h post injection.

[0022] FIG. 5 illustrates the general structure of the water-soluble polymeric delivery system. The average mol percentage of targeting moieties (T) per polymer chain may range anywhere from 0 % to about 50 %, preferably from 0 % to 30 %; The average mol percentage of therapeutic agents or imaging agents (D) or mixture of

both per polymer chain may range anywhere from 1 % to about 90 %; The average mol percentage of bio-assay label (L) per polymer chain may range anywhere from 0 % to about 50 %. The spacer S_1 and S_2 can be covalent or physical bonds or linkages, such as peptides or other complex chemical structures, which may or may not be cleaved upon stimulus, such as change of pH, specific enzyme activity (for example, cathepsin K, MMPs, etc.), presence or absence of oxygen, etc. under physiological condition. The spacer S_3 illustrates a non-degradable, under physiological condition, covalent or physical bond or linkage. The optional biodegradable cross-linkage (C) can be covalent or physical bonds or linkages, such as peptides or other complex chemical structures, which may be cleaved upon stimulus, such as a change of pH, specific enzyme activity (*e.g.*, cathepsin K, MMPs, etc.), presence or absence of oxygen, etc., under physiological condition.

[0023] FIG. 6. illustrates the chemical structure of a exemplary polymeric drug delivery system, with dexmethasone as an example of a therapeutic agent, for the treatment of arthritis. The polymeric prodrug is abbreviated as P-Dex.

[0024] FIG. 7. shows the superior therapeutic effect of P-Dex over dexamethasone sodium phosphate (Dex).

DETAILED DESCRIPTION OF THE INVENTION

[0025] Numerous reports have indicated abnormally high plasma protein concentration in the affected joints of RA patients (4, 5, 22), which support the inventions use of macromolecular delivery systems to specifically target anti-inflammatory drugs or non-invasive imaging agents to diseased joints. Such targeting may help to achieve superior therapeutic effects or better imaging and evaluation of the diseased sites.

[0026] To demonstrate the principle of the invention, conventional visual examination with Evans blue dye (EB) injection and magnetic resonance imaging (MRI) techniques were used to follow the *in vivo* fate of macromolecules on an established AIA rat model. Additionally, histological examination confirmed the presence of disease in specific anatomical locations where the MRI technique identified the macromolecular delivery system.

[0027] EB is a commonly used agent to assess vascular permeability and integrity (23). It is a dye-carrying multiple charges and aromatic structures, which forms

a strong complex with plasma albumin. Injection of the dye had been successfully used to establish the concept of macromolecular therapy for the treatment of solid tumors (24). In the present study, we used the EB dye technique in AIA rats to visually assess the accumulation of plasma albumin in inflamed joints. The hind paw of the AIA rats, where the most severe inflammation was evident, readily incorporated the dye compared with that observed in the healthy rats. This observation confirmed that there was indeed a much greater concentration of plasma albumin in the inflamed joints of the AIA rat model.

[0028] Although the results with EB are significant, it is noted that the dye is not covalently bound to albumin and some dye transfers nonspecifically to other tissues. For example, a slight blue staining was evident in some organs, including the liver and heart.

[0029] Magnetic resonance imaging (MRI) is a noninvasive method of mapping the internal structure of the body. It employs radiofrequency (RF) radiation in the presence of carefully controlled magnetic fields in order to produce high quality cross-sectional images of the body in any plane. It portrays the distribution of hydrogen nuclei and parameters relating to their motion in water and lipids. Introduction of paramagnetic contrast agents would shorten T_1 (the longitudinal relaxation time) of the hydrogen nuclei in tissues, which in turn will increase the MR signal intensity thereof (14). Therefore, to further support the results, magnetic resonance imaging (MRI) was used to track the DOTA-Gd^{3+} labeled macromolecules injected in AIA rats.

[0030] It is well understood that the introduction of paramagnetic contrast agents shortens the longitudinal relaxation time (T_1) of the hydrogen nuclei in tissues (25). The relation between the contrast agent concentration I and the T_1 value can be expressed in the following formula, where T_{10} is the initial T_1 relaxation time and r_1 is the relaxivity. Apparently, the increase of contrast agent concentration C would lead to the reduction of T_1 value.

[0031] $$\frac{1}{T_1} = \frac{1}{T_{10}} + r_1 C$$

[0032] The relation between T_1 and the signal intensity (I) in the MR imaging is rather complicated. This relation can be expressed as:

[0033] $$I = M_0 \frac{(1-E)\sin(\theta)}{(1-E)\cos(\theta)}$$

[0034] where $E = e^{-\frac{T_R}{T_1}}$, T_R is repetition rate of the imaging sequence and M_0 is the local equilibrium magnetization density. For large tip angles (θ = 25° or greater) and short T_1, this expression can be approximated as:

[0035] $$I = K(1-E) \text{ or } I = K(1-e^{-\frac{T_R}{T_1}})$$

[0036] Apparently, as T_1 gets shorter, the signal intensity (I) increases.

[0037] Therefore, correlation of the above two equations (**[0030]** and **[0034]**) will provide a qualitative understanding that the higher MR contrast signal intensity in the MR images obtained represent the existence of a higher concentration of the paramagnetic contrast agents in the tissue. The analysis of the macromolecular contrast agents enhanced MR images of the rats provides important information about the pharmacokinetics profile and biodistribution of the water-soluble polymeric delivery systems described in this invention. Thus, an imaging agent may enhance the resolution of an imaging process, which is used to generate an image of a subject (preferably a mammal, such as a human), an animal (including, an animal model for a particular disease), or part (*e.g.*, a tissue or structure) of the subject or animal.

[0038] Conjugation of a low molecular weight paramagnetic contrast agent, DOTA-Gd^{3+} complex to HPMA copolymer enabled the non-invasive monitoring of the fate of the injected polymer in rats with MR scanner. This approach of labeling the polymer with a MRI contrast agent is similar to labeling the polymers with fluorochromes to permit localization in organs, tissues and cells. A similar approach can also be used with other imaging agents for PET, CT and γ-scintigraphy for the purposes of non-invasive imaging and evaluation of the diseased tissues and organs.

[0039] Therefore, a macromolecular magnetic resonance imaging (MRI) contrast agent based on N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer has been synthesized to illustrate the invention. After systematic administration of the contrast agent in an adjuvant induced arthritis (AIA) rat model, contrast enhanced MR images were taken, which show the distribution of the polymer at different time points. Correlating the MR results with additional visual and histopathological results from the

AIA rats', demonstrates the preferential deposition and retention of macromolecules in the inflamed joints. Thus, demonstrating the effectiveness of using macromolecular therapy for the treatment of rheumatoid arthritis. In addition, these results demonstrate the feasibility of using macromolecular imaging agents for imaging and evaluation of arthritic joints.

[0040] The invention includes polymeric delivery systems for the delivery of drugs, such as anti-inflammatory drugs.

[0041] The invention includes polymeric delivery systems for the delivery of imaging agents, such as chemical compounds used as enhancing agents in MRI (for example, DOTA-Gd^{3+}, DTPA-Gd^{3+}, etc.), PET (for example, compounds labeled or complexed with ^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{64}Cu, ^{68}Ga, ^{82}Rb, etc., such as ^{18}F-FDG), CT (for example, Iodine or barium containing compound, such as 2,3,5-triiodobenzoic acid) and γ-scintigraphy (for example, compounds complexed with ^{99}Tc, ^{111}In, ^{113}In, and ^{153}Sm, etc.) imaging.

MRI procedure

[0042] MR images of the animals were acquired on a 1.5 T Signa LX imaging system (General Electric Medical Systems, Milwaukee, WI), using a phased-array coil. Images were acquired using a 3D single slab IR prepped FSPGR sequence in the coronal plane. The common imaging parameters were TR = 13.4 ms, TE = 2.2 ms, TI = 300 ms, 25 ° flip angle, 512 X 256 in-plane acquisition matrix, 20 X 10 cm^2 field-of-view (FOV), 64 slices per slab, 1.0 mm thick slices with 2 X interpolation to 0.5 mm.

Synthesis of poly(HPMA-co-APMA-co-MA-FITC).

[0043] HPMA (1 g, 7 mmol), APMA (0.14 g, 0.78 mmol), MA-FITC (0.043 g, 7.8 mmol), AIBN (0.057 g, 0.35 mmol) and MPA (0.001 mL, 1 mmol) were dissolved in methanol (10 mL), placed in an ampoule and purged with N2 for 5 min. The ampoule was flame-sealed and maintained at 50 °C for 24 hrs. The polymer was isolated by precipitation of the resulting solution into acetone and was reprecipitated twice. After the polymer was dried in desiccator (over NaOH), the final yield was determined as 0.9 g.

The content of free amino groups in the copolymer was determined as 7.7 X 10^{-4} mol/g using the ninhydrin assay (18).

Synthesis of P-DOTA

[0044] Poly(HPMA-co-APMA-co-MA-FITC) (170 mg, $[NH_2]$ = 1.33 x 10^{-4} mol), DOTA-NHS ester (100 mg, 2 x 10^{-4} mol) and diisopropylethyl amine (DIPEA, 160 mL, 9.33 x 10^{-4} mol, distilled from ninhydrin) were mixed in DMF (1.5 mL, distilled from P_2O_5) and stirred overnight. The conjugate was precipitated into ether and dried in vacuum. The product was further purified on LH-20 column, dialyzed (molecular weight cutoff size is 6-8 kDa) and lyophilized to obtain 190 mg of final product. The residue free NH2 group was determined with ninhydrin assay and the content of DOTA in the product was calculated as 7.5 x 10^{-4} mol/g.

Synthesis and purification of macromolecular MRI contrast agent P-DOTA-Gd^{3+}

[0045] P-DOTA (100 mg, [DOTA] = 6.9 x 10^{-5} mol) and $GdCl_3 \cdot 6H_2O$ (38 mg, 1.04 x 10^{-4} mol) were dissolved in 2 mL deionized H_2O. The pH of the solution was maintained at 5.0-5.5 over night by gradual addition of NaOH (1 N) solution. EDTA disodium salt (38 mg, 1.04 x 10^{-4} mol) was then added into the solution to chelate the excess Gd^{3+}. After stirring for 30 min, the milky solution was purified with Sephadex G-25 column to remove the EDTA-chelated Gd^{3+} and other unreacted low molecular weight compounds from the polymer conjugate. The conjugate was lyophilized to yield 115 mg P-DOTA-Gd^{3+}. The gadolinium content was determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) as 0.52 mmol/g. The Mw of the polymeric MRI contrast agent is determined FPLC as 55 kDa with a polydispersity of 1.43. The T_1 relaxivity of the conjugate was determined as 10.4 $mM^{-1}s^{-1}$ per complexed Gd^{3+} using a B1 homogeneity corrected Look-Locker technique on the 1.5T GE NV/Cvi scanner with the LX 8.4 operating system at room temperature (19). The chemical structure of the macromolecular MRI contrast agent is shown in FIG. 1.

Synthesis of P-Dex

[0046] HPMA (1 g, 0.00698 mol), MA-GG-OH (0.156 g, 0.00078 mol), MA-FITC (0.02 g, 0.00004 mol) and AIBN (0.007 mg, 0.000043 mol) were dissolved in DMSO (1 mL) and MeOH (8 mL) mixture. The solution was transferred into an ampoule and purged with N_2 for 5 min. Then polymerized at 50 °C for 24 h. The polymer was then reprecipitated twice to yield about 1 g of copolymer. It was further reacted with a large excess of hydroxy succinimide (HOSu) and hydrazine. After reprecipitation, dexamethasone was conjugated to the copolymer in the presence of 1 drop of acetic acid in DMF. The conjugate was purified with LH-20 column and freeze-dried to obtain the final conjugate (structure shown in FIG. 6) with dexamethasone content of 49 mg/g (of conjugate). The Mw of the conjugate is 73 kDa.

Adjuvant induced arthritis rat model

[0047] Male Lewis rats (175-200 g) were obtained from Charles River Laboratories (Portage, MI) and allowed to acclimate for at least one week. To induce arthritis, Mycobacterium Tuberculosis H37Ra (1 mg) and LA (5 mg) were mixed in paraffin oil (100 μL), sonicated and s.c. injected into the base of the rat's tail (20). The rats were then randomized into 3 rats/group. The progression of the joint inflammation was followed by measuring the diameter of the ankle joint with calipers. Special care was given to the rats as the inflammation developed to ensure availability and access to water and food. The MRI contrast agents used for the study were injected directly into the jurgular vein while the animal was anesthetized with Ketamine and Xylazine.

Visualization of plasma albumin accumulation in RA joints

[0048] Evans blue dye (EB, 10 mg/kg in saline) was injected into healthy and AIA rats via the tail vein. The extravasation and accumulation of dye in the areas of joint inflammation could be visually observed as appearance of the blue pigment. Photographs of the ankle and paws were taken before and 8 h after injection.

Histology

[0049] At necropsy, the major organs and limbs were removed and fixed with 10% phosphate buffered formalin for 24 h. The organs were then dehydrated and

embedded in paraffin for routine histopathological analyses. The limbs were gradually dehydrated in ascending concentrations of ethanol and embedded in poly(methyl methacrylate). Sections of the entire joint, including the undecalcified bone, were cut with a low speed saw using diamond-wafering blades. The sections were mounted to plastic slides, ground to about 50 μm in thickness and surface stained using a Giemsa stain modified for plastic sections (21). The joints (knee, ankle, tarsals and metatarsals) from the same animals that were imaged by MRI were assessed for the presence of inflammation and tissue damage using the histology sections.

Visual and histological examination of AIA rats

[0050] The development of adjuvant-induced arthritis in the rat is well described in the literatures (20), and briefly summarized here. After injection of the adjuvant, changes begin to become evident about 9 days later. This includes some inflammation around the eyes and enlarged and tender external genitalia. Inflammation and swelling of the front and hind limb ankle joints becomes evident at about 12 days after injection of the adjuvant.

[0051] At necropsy at 15 days post injection of the adjuvant, inflammation of the peritoneum (peritonitis) can be observed. Occasionally, inflammation of gastrointestinal (GI) tract and fluid retention in the peritoneal cavity are also detectable. Grossly, most of the vital organs appear to be normal except that the spleen is usually enlarged with visual evidence of inflammation.

[0052] However, under histopathological examination, all organs examined showed signs of chronic inflammation. The testicular tissue demonstrates inflammation in the membranes around the testis, with small granulomata in the epididymis being detected. The pericardial tissue demonstrates chronic inflammation, which easily could allow for build up of fluid in the pericardial tissue. The renal tissue includes multifocal areas of granulomata formation in the cortical tissue with some inflammation over the capsule, particularly along potential serosal surface changes. The splenic tissue demonstrates multifocal areas of necrosis surrounded by neutrophils and epithelioid cells. Plasma cells and lymphocytes are responding around this process, which indicate a rather severe inflammatory response throughout the splenic tissue.

[0053] The histological images of the AIA rats' hind legs are presented in FIG. 2. At lower magnification, the swelling of the ankle joint region and paws of the AIA rats is evident (FIG. 2A). Extensive inflammation, synovitis, bone and cartilage destruction is evident (FIGs. 2B to 2D). Inflammatory cells are observed around the larger vessels (FIG. 2E). By contrast, the knee joints from the AIA rats are typically less affected by the inflammatory process (FIG. 2F).

Visual examination of the AIA rats after Evans blue injection

[0054] For this experiment, EB was injected into the rats 15 days after injection of the adjuvant. By this time, there is a robust inflammatory reaction evident in the ankle joints. After injection of EB, there was a gradual accumulation of blue color in the inflamed hind paw and front paws of the AIA rats with high density of the color located around tarsus and carpus. Some deep blue spots were also observed on some digits of the paw. Photographs taken before and after injection of the EB dye confirm that the areas with dye accumulation correspond to those with marked inflammation. In the healthy control rats given EB, the dye was not localized to joint areas as observed in the AIA rats.

MR imaging

[0055] Imaging AIA rats with P-DOTA-Gd^{3+} as a contrast agent. Immediately prior to the injection of the P-DOTA-Gd^{3+} contrast agent, a baseline MRI scan was done. The animals were then injected with the contrast agent and MRI scans were performed at different time intervals. The acquired images were post processed using the maximum intensity projection (MIP) algorithm. The resulting MIP images of the animals are depicted chronologically in FIG. 3.

[0056] As shown in the baseline image (FIG. 3A) before contrast injection, the intestine and stomach of the animal are clearly visible likely due to fluid retention. Several irregular spots are also observed in the lower abdomen, which can be attributed to i.p. injection site(s) of anesthetic agents. An area in the scrotum, adjacent to the testes in the anatomical region of the epididymus and associated tissue also shows a diffuse MR signal, perhaps due to its fatty content or accumulation of fluid. The bright spot at the

right sciatic region may represent the fluid retention in an inconsistent lymph node called lc. Ischiadicum, which is also evident is some of the subsequent images from this animal. No significant MR signal was observed at the hind limbs. The ankle joints were, however, clearly enlarged in the latent image when compared with similar images of the controls.

[0057] At five minutes after the injection of the macromolecular contrast agent, there was substantial MR signal in the kidneys (FIG. 3B). A detailed examination of the single-plane 2-dimensional images indicates that at this time most of the contrast is in the kidney cortex with little in the medulla. Because of the overall increase of the image contrast after the injection, the bladder became evident as a negative image (dark) as is the oval shaped structure at lower left abdominal area. Increased contrast is also observed in the liver, spleen and bone marrow. The major blood vessels are clearly defined while the lesser vessels are not as apparent, probably due to the limited imaging resolution (about 0.5 mm) with the 1.5 T MRI scanner. However, the vessels appear larger, perhaps dilated, than those observed in the healthy controls. Except for some uptake in the bone marrow, little significant contrast signal was evident at this time in the inflamed ankle region.

[0058] In the MR images (FIG. 3C) of the AIA rats acquired 1 h post injection, the signal in the cortex of kidneys was greatly reduced compared with the earlier (5 minute) time. However, now most of the contrast appears to be concentrated in the kidney medulla and pelvis. Both ureters contain contrast material and a substantial signal is now evident in the urinary bladder. There appeared to be slight decrease in the MR contrast signal in the liver, spleen and blood vasculature. Interestingly, several "hot spots" start to appear around tarsus, where the most severe inflammation occurs in this animal model.

[0059] From the MR images acquired 2 h (FIG. 3D) and 3 h (FIG. 3E) post injection of the macromolecular contrast agent, a gradual reduction of MR contrast signal was evident in the kidney (cortex and medulla), liver, spleen and vasculature. There was, however, an accumulation of the contrast material in the urinary bladder. The "hot spots" detected around tarsus at the 1 h scan continue to expand and increase in contrast in the 2 h (Figure 4-B) and 3 h images.

[0060] When the rats were scanned again at 8 h post injection, the MR images (FIG. 3F) acquired show greatly reduced MR signal in all the vital organs and blood vessels with essentially an undetectable bladder, even though the overall body signal remains slightly greater than that of the baseline images. Surprisingly, however, the MR contrast signal is substantially increased in the ankle joint and metatarsal region (FIG. 4C). The initial "hot spots" disappear and the MR contrast signal is more evenly distributed around the joint tissue. Also observed is some contrast signal in the posterior knee joints, but with a much less intensity and size (FIG. 4 D).

[0061] Subsequently, the animals were again scanned at 32 h and 48 h post injection, respectively (FIGs. 3G and 3H). The overall contrast enhancement of MR signal continued to decline from that observed in the 8 hr images. However, the decrease in image contrast in the ankle joint tissue appeared to be much slower than observed in other tissues and organs. Even after 48 h, the enhancing effect of the injected macromolecular contrast agents is still visible in the hind ankle and paw tissue.

[0062] Imaging healthy rats with P-DOTA-Gd^{3+} as a contrast agent. In the MR images (FIG. 3J) taken 5 min after the injection of macromolecular contrast agent, the kidneys of the healthy animals showed extremely strong contrast signal. The single-plane 2-D images indicate that the MR contrast resides in both the cortex and medulla. Both side ureters are partially visible. The urinary bladder is filled with a significant amount of contrast medium. Liver, spleen and bone marrow were visible in the image when compared with the baseline image. The major blood vasculatures, including abdominal aorta and inferior vena cava were also highlighted. The arrangement and appearance of these vessels appears to be normal. No contrast signal was detected outside of the large vessels in the hind paws.

[0063] In the MR images taken at 1 h (FIG. 3K) and 2 h (FIG. 3L) post injection, little contrast media remains in the kidney cortex and medulla, but some contrast signal remained in the kidney pelvis and bladder. The contrast enhancement of the vasculature was slightly reduced compared with that observed at 5 minutes after injection. At 8 h (FIG. 3M) after injection, the contrast media was completely cleared from the urinary tract. At this time, some of the large vessels were still evident, though less so than at earlier times. The images taken at 48 h (FIG. 3N) after injection replicate

the baseline images with no detectable contrast enhancement. As expected, all MR images taken at different times post injection did not show contrast enhancement in the hind-limb joints of the animal.

Imaging AIA and healthy rats with OMNISCAN as contrast agent.

[0064] The images acquired with the MR enhancement of a low molecular weight paramagnetic contrast agent OMNISCAN (gadolinium complex of diethylenetriamine pentaacetic acid bismethylamide) were obtained similarly as those injected with P-DOTA-Gd^{3+}.

[0065] From the images sequence in FIG. 3 (3O to 3X), a very fast overall tissue contrast enhancement at 5 min post injection in healthy rats (FIGs. 3O & 3T) was observed in both healthy and AIA rats. However, the contrast enhancement quickly declined, accompanied by a rapid renal clearance of the contrast medium. At 8 h (FIGs. 3Q & 3W), the enhancement was basically gone. Interestingly, the 5 min images (FIG. 3O) of the AIA rats reveal significant contrast enhancement at the inflamed ankle joints, which had cleared at the 2 h scan (FIG. 3P). However, no such observation was found in the healthy rats. Basically, no blood vasculature contrast enhancement could be observed in all OMNISCAN enhanced MR images.

[0066] As shown in FIG. 3, all vital organs in AIA rats showed greater uptake of P-DOTA-Gd^{3+} than the healthy rats. In addition, the clearance of the contrast agent in these organs was slower than those in healthy rats, especially in the kidneys. These observations are consistent with the histological findings that all organs in AIA rats, including heart, liver, lung, kidney and spleen had some granulomatous chronic inflammation. The vasculature in such inflamed tissues is often more porous, permitting a greater extravasation of macromolecules to the interstitial tissue. These may lead to organ dysfunction, such as the delayed renal clearance of the polymer contrast agent compared to healthy rats. However, the major clearance of P-DOTA-Gd^{3+} from these organs was completed within a few hours (< 8 h) in the AIA rats. When compared with normal rats, the major blood vessels appear to be dilated in the AIA rats. This observation may be due to the up-regulated prostaglandins level in this systematic inflammation model (26). It may also help to explain the observed faster polymer extravasation.

[0067] Interestingly however, extravasation in the inflamed ankle joints was delayed for a short time (1~2 h) in the AIA model (FIGs. 3A-3H). The "hot spots" of high MR contrast signal appeared later around the tarsus indicating high local concentrations of P-DOTA-Gd^{3+}. These "hot spots" also reveal the locations of possible local damage in and around the joint. The polymer continues to extravasate, diffuse, accumulate in the ankle joints and the greatest concentrations were observed in the 8 h post injection images (single plane, enlarged MR images, FIG. 4). Because some increased concentrations of polymer were still observed in the joint at 32 h after injection, it appears that the clearance of the polymer from the joint is relatively slow. By correlating the polymer accumulation, as detected by MRI, with the histology of the same tissues (FIGs. 2A, 2B and 2D), it is evident that the accumulation of the polymer correlates with the degree of inflammation. As observed in the 8 h MR images, the accumulation of P-DOAT-Gd^{3+} to the knee joints was much less than that observed to the ankle joints (FIG. 4D). This finding agrees very well with the amounts and degree of severity of inflammation observed histologically in the joints (FIG. 2F). In contrast to the observation with AIA rats, no extravasation of P-DOTA-Gd^{3+} to the ankle or knee joints was observed in the healthy control rats.

[0068] The data suggests a pharmacokinetic profile with a renal clearance mechanism and a redistribution of the HPMA copolymer (labeled with DOTA-Gd^{3+}) from major organs and the blood circulation compartment into the inflammatory arthritic joints. Compared to the normal animal, the result from the MR images of the AIA model clearly demonstrate a very specific polymer targeting and accumulation effect to the arthritic joints with a time frame of about 1 to 2 days after a single bolus injection. Given that most current anti-arthritic drugs do not specifically target the arthritic joints and the damaged tissues, coupled with a low efficacy, the observed targeting and accumulation of the polymeric delivery systems to arthritic joints demonstrate the great effectiveness and numerous potential applications of this invention for the drug delivery and treatment, for example, of rheumatoid arthritis.

[0069] Likewise, imaging and evaluation of the inflammatory tissues or organs, such as arthritic joints, with an MRI macromolecular contrast agent, also provides much improved imaging results, as shown in FIGs. 3F & 4C, when compared to the low

molecular weight MRI contrast agent, such as OMNISCAN (FIG 3O). The invention permits a greater time frame for longer and/or more detailed and/or sophisticated imaging process, which can't be, or are not optimally, performed with the current low molecular weight imaging agents, such as OMNISCAN. More anatomical detail can be revealed with these imaging agents, which may have many applications, such as preclinical evaluation of therapeutic effects of experimental anti-arthritic drugs on an animal model and clinical evaluation of patient response to treatment. When MRI, PET, CT or γ-scintigraphy imaging agents are conjugated to the polymeric delivery systems described in this invention, they will be able to provide powerful molecular imaging tools for the understanding of inflammatory diseases, such as rheumatoid arthritis.

[0070] While the enhanced permeability of the vasculature in the arthritic joints may be comparable to those found in solid tumor, the retention of the polymer in the joint tissue may be weaker, as evidenced by the shorter accumulation time. The shorter accumulation time may be attributed to elevated intraarticular pressure (27) and the existence of lymphatic vessels, although they might have been partially damaged or deformed (6, 7). Therefore, the polymeric anti-arthritic drug delivery may be modified relative to those used in the treatment of a solid tumor.

[0071] Due to the nature of a short polymer accumulation time, a swift drug-cleavage mechanism may be applied to ensure effective release of the drug from the macromolecular carrier. A person of ordinary skill in the art will recognize that some pathological features of the arthritic joints may be exploited for this. For example, the release of the drug from the polymer may be facilitated by things such as the very high extracellular enzyme activities (*e.g.*, cathepsin K, MMPs, etc.) (28), low pH, hypoxia or elevated temperature (29). Likewise, measures that would enhance the retention of the extravasated polymers in the joints may also be used according to the invention (*e.g.*, the polymer drug conjugates). Incorporation of targeting moieties, which would bind to the negatively charged cartilage (30), the freshly eroded bone surface (21) or the enriched rheumatic factors in the RA joints may also be used to increase the uptake and retention of the polymer in joint tissue. It is also believed that by increasing the molecular weight of the polymeric carrier, a greater retention of the polymer in the RA joint may be

accomplished. Anti-arthritic drugs with fast therapeutic effects, such as glucocorticoids, can be used in the drug delivery system of the invention.

[0072] In addition, currently available protein drugs and orally available low molecular weight drug may also benefit from the principles illustrated in the invention. For example, the extravasation of the injected polymer into the RA joints was delayed for 1 to 2 h. Thus, for the protein or peptide drugs, they must survive this period of time against hepatic and renal clearance. PEGylation of the protein is believed to provide a beneficial means in solving this problem (3). Currently, screening of low molecular weight drug candidates seems to be largely dependent on the therapeutic potency they demonstrated in *in vitro* assays. However, current studies suggest that the albumin binding capacity may be another important factor one must consider in drug candidate selection (32). A molecule, which has strong albumin binding ability, may be beneficially used in the invention to provide a longer half-life and higher local bioavailability in the RA joints. In this case, the plasma albumin may be considered as its natural macromolecular carrier, which could take the advantage of the joint-specific accumulation effect we observed in the study.

[0073] Using modern MR imaging techniques, we observed the specific accumulation of macromolecules in arthritic joints in adjuvant-induced arthritis in rats. There was an excellent correlation between the uptake and retention of the MR contrast agent labeled polymer with histopathological features of inflammation and local tissue damage. The methodology used in this study proved that macromolecular imaging agents (polymeric delivery systems conjugated with MRI, CT PET, γ-scintigraphy imaging agents) are powerful imaging and evaluation tools for inflammatory diseases, such as rheumatoid arthritis. Based on the same evidence, the macromolecular drug delivery systems of the invention provide a beneficial improvement of current treatments, for example, for rheumatoid arthritis. The invention provides the ability to increase the therapeutic potential and dosing window of the drugs by reducing their side effects. Furthermore, the invention may have a longer half-life in blood circulation when compared to low molecular weight drugs, which may increase the bioavailability of the drug. In addition, the invention may be used to render a hydrophobic drug hydrophilic and, particularly for peptide-based drugs, reduce immunogenecity.

[0074] To demonstrate the superior therapeutic effects of the invention, a HPMA copolymer containing targeting moiety with an anti-arthritic drug was synthesized. Hydrazine was used as the targeting moiety, as it may bind to negative charged moieties on cartilage. The anti-arthritic drug, dexamethasone, was linked to the polymer backbone (P-Dex) via a pH sensitive hydrozone bone as illustrated in FIG. 6. The polymer with the hydrazine and dexamethasone attached was then injected into AIA rats (4/group) on day 13 after the induction of arthritis. A single dose of 10 mg (P-Dex)/kg was given. As a control, the same dose of low molecular weight Dexamethasone sodium phosphate (Dex) was divided into 4 equal doses and one dose was given each day to another group of AIA rats (4/group) from day 13-16 after the induction of arthritis. As shown in FIG. 7, both groups of animals showed a dramatic decrease of ankle joint swelling after the injections on day 13. However, with the cessation of the daily injections of the control Dex, the inflammation rapidly got worse while the inflammation in the P-Dex group had a prolonged suppression. These significant advantages of the P-Dex treatment may be attributed to the specific targeting and enhanced retention (because of the cartilage targeting moiety) of the polymeric delivery system to the arthritic joints of the animals.

[0075] A water-soluble polymer backbone of the invention includes, but is not limited to, a HPMA copolymer and its derivatives, polyethylene glycol (including branched or block copolymers, which may be degradable via peptide sequences, ester or disulfide bonds, etc.), polyglutamic acid, polyaspartic acid, dextran, chitosan, cellulose and its derivatives, starch, gelatin, hyaluronic acid and its derivatives, polymer or copolymers of the following monomers: *N*-isopropylacrylamide, acrylamide, *N,N*-dimethylacrylamide, *N*-vinylpyrrolidone, vinyl acetate (resulting polymer hydrolyzed into polyvinyl alcohol or PVA), 2-methacryloxyethyl glucoside, acrylic acid, methacrylic, vinyl phosphonic acid, styrene sulfonic acid, maleic acid, 2-methacrylloxyethyltrimethylammonium chloride, methacrylamidopropyltrimethylammonium chloride, methacryloylcholine methyl sulfate, *N*-methylolacrylamide, 2-hydroxy-3-methacryloxypropyltrimethyl ammonium chloride, 2-methacryloxyethyltrimethylammonium bromide, 2-vinyl-1-methylpyridinium bromide, 4-vinyl-1-methylpyridinium bromide, ethyleneimine, (*N*-acetyl)ethyleneimine, (*N*-

hydroxyethyl)ethyleneimine and/or allylamine. Preferably, the water-soluble polymer is biologically inert, however, optionally the polymer may have therapeutic activity (31).

[0076] The invention may, optionally, include one or more targeting moieties, which may be used to direct the delivery system to a specific tissue, such as bone, cartilage, etc. Illustrative examples of targeting moieties include, but are not limited to, bisphosphonates, quaternary ammonium groups, peptides (*e.g.*, oligo-Asp or oligo-Glu), aminosalicylic acid, and/or antibodies or fragments or derivatives thereof (*e.g.*, Fab, humanized antibodies, and/or scFv). A targeting moiety may be linked to the polymer backbone via covalent or physical bonds (linkages). Optionally, the spacers between a targeting moiety and the polymer backbone may be cleaved upon a stimulus including, but not limited to, changes in pH, presence of a specific enzyme activity (for example, cathepsin K, MMPs, etc.), changes in oxygen levels, etc.

[0077] Optionally, the spacers between the therapeutic agent and the polymer backbone may be cleaved upon a stimulus including, but not limited to, changes in pH, presence of a specific enzyme activity (for example, cathepsin K, MMPs, etc.), changes in oxygen levels, etc.

[0078] Optionally, a bio-assay label (or labels) may be attached to the polymer backbone. It may be any label known in the art, including, but not limited to, a radioisotope, biotin, gold, etc. Their average mol percentage per polymer chain may range from 0 % to about 50 %.

[0079] The bio-assay label, therapeutic agent, and/or targeting moiety may be linked to the water-soluble polymer backbone by way of a spacer. Spacers are known in the art and the person of ordinary skill in the art may select a spacer based on length, reactivity, flexibility and the like. For example, a spacer may be an alkyl or alkyne having from one to 50, preferably one to 15 carbons.

[0080] A spacer of the invention may be a peptide sequence (for example, selected from all nature amino acids) having from one to 20, preferably one to 10 residues. In yet another example, a spacer may contain a hydrozone bond which is cleavable under acidic pH. These spacers may be cleaved upon a stimulus including, but not limited to, changes in pH, presence of a specific enzyme activity (for example, cathepsin K, MMPs, etc.), changes in oxygen levels, etc.

[0081] Optionally, the biodegradable cross-linkage shown in FIG. 5 may cross-link, to a certain degree, the linear polymer backbone. The resulting delivery system still retains its water-solubility. The linkage itself is preferably cleavable under physiological conditions.

[0082] As will be appreciated by a person of ordinary skill in the art, each class (*e.g.*, therapeutic agent, targeting moiety, bio-assays label, spacer and/or imaging agent) may comprise any number of different compounds or compositions. For example, the therapeutic agent may consist of a mixture of one or more NSAIDs and one or more glucocorticoid, such as a combination of dexamethasone and hydrocortisone. Therefore, the invention provides the advantage that any combination of different therapeutic agents, targeting moieties, bio-assays labels, spacers and/or imaging agents may be incorporated onto the water-soluble polymer backbone. As a result, a drug delivery or imaging system can be created with two or more different therapeutic agents and/or two or more different targeting moieties and/or two or more different bio-assays labels, and/or two or more different spacers (one or more of which may be cleavable, wherein the cleavage stimulus may be different for different spacers) and/or two or more imaging agents.

[0083] An effective amount of a drug is well known in the art and changes due to the age, weight, severity of a subject's condition, the particular compound in use, the strength of the preparation, and the mode of administration. The determination of an effective amount is preferably left to the prudence of a treating physician, but may be determined using methods well known in the art (37, 38). The compositions of the invention may be prepared using methods known in the art, for example, the preparation of a pharmaceutical composition is known in the art (37, 38).

[0084] The compositions may be administered by any desirable and appropriate means. For *in vivo* delivery (*i.e.*, to a subject having arthritis or other inflammatory diseases), it is preferred that the delivery system be biocompatible and preferably biodegradable and non-immunogenic. In addition, it is desirable to deliver a therapeutically effective amount of a compound in a physiologically acceptable carrier. Injection into an individual may occur intravenously, intramuscularly, or, for example, directly into a localized area (*e.g.*, synovial fluid). Alternatively, *in vivo* delivery may be

accomplished by use of a syrup, an elixir, a liquid, a tablet, a pill, a time-release capsule, an aerosol, a transdermal patch, an injection, a drip, an ointment, etc.

ABBREVIATIONS

[0085] AIA, adjuvant induced arthritis; AIBN, 2,2'-azobisisobutyronitrile; APMA, N-(3-Aminopropyl)methacrylamide hydrochloride; COX-2, cycloxygenase-2; CT, computerized tomography; Dex, Dexamethasone sodium phosphate; DMARDs, disease-modifying antirheumatic drugs; DIPEA, diisopropylethyl amine; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(acetic acid); DOTA-NHS ester, 1,4,7,10-tetraazacyclododecane-1,4,7-tris(acetic acid)-10-acetic acid mono (N-hydroxysuccinimidyl ester); DTPA-Gd^{3+}, gadolinium complex with diethylenetriamine pentaacetic acid; EB, Evans blue; EPR, enhanced permeability and retention; ^{18}F-FDG, fluorodeoxyglucose; FITC, fluorescein isothiocyanate; FPLC, fast protein liquid chromatography; HPMA, N-(2-hydroxypropyl)methacrylamide; ICP-OES, inductively coupled plasma optical emission spectroscopy; IL-1Ra, interleukin-1 receptor antagonist; LA, N,N-dioctadecyl-N',N'-bis(2-hydroxyethyl) propanediamine; MA-FITC, N-methacryloylaminopropyl fluorescein thiourea; MA-GG-$NHNH_2$, N-methacryloyl glycylglycyl hydrazine; Mn, number average molecular weight; MPA, mercaptopropionic acid; MRI, magnetic resonance imaging; Mw, weight average molecular weight; NSAIDs, symptomatic treatment with non-steroidal anti-inflammmatory drugs; OA, osteoarthritis; OMNISCAN, or gadodiamide is the injectable formulation of the gadolinium complex of diethylenetriamine pentaacetic acid bismethylamide; P-Dex, conjugate of dexamethasone to copolymer of HPMA, MA-GG-$NHNH_2$ and MA-FITC via hydrozone bond (FIG. 6.); PET, positron emission tomography; PHPMA, poly[N-(2-hydroxypropyl)methacrylamide]; Poly(HPMA-co-APMA-co-MA-FITC), copolymer of HPMA, APMA and MA-FITC; P-DOTA, conjugation product of poly(HPMA-co-APMA-co-MA-FITC) and DOTA-NHS ester; P-DOTA-Gd^{3+}, purified complex of P-DOTA and Gd^{3+}; RA, rheumatoid arthritis; R.T., room temperature; SEC, size exclusion chromatography; scFv, single chain variable fragment; TMJ, temporomandibular joint syndrome; TNF, tumor necrosis factor.

[0086] All references, including publications, patents, and patent applications, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein. The following list of references are hereby incorporated by reference:

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[00125] While this invention has been described in certain embodiments, the present invention can be further modified within the spirit and scope of this

disclosure. This application is therefore intended to cover any variations, uses, or adaptations of the invention using its general principles. Further, this application is intended to cover such departures from the present disclosure as come within known or customary practice in the art to which this invention pertains and which fall within the limits of the appended claims.

CLAIMS

What is claimed is:

1. A pharmaceutical composition for the treatment of an inflammatory disease comprising:

   a water-soluble polymer and an effective amount of a therapeutic agent linked to said water-soluble polymer, wherein the therapeutic agent is used in the treatment of an inflammatory disease.

2. The pharmaceutical composition of claim 1, further comprising a targeting moiety linked to the water-soluble polymer.

3. The pharmaceutical composition of claims 1 or 2, wherein the inflammatory disease comprises arthritis.

4. The pharmaceutical composition of claims 1, 2 or 3, wherein the water-soluble polymer is selected from the group consisting of a HPMA copolymer, polyethylene glycol, polyglutamic acid, polyaspartic acid, dextran, chitosan, cellulose, starch, gelatin, hyaluronic acid and derivatives thereof.

5. The pharmaceutical composition of any one of claims 1-4, further comprising a bio-assay label linked to the water-soluble polymer.

6. The pharmaceutical composition of any one of claims 1-5, further comprising a spacer between the therapeutic agent and the water-soluble polymer, wherein the spacer is cleavable.

7. The pharmaceutical composition of any one of claims 1-5, further comprising a spacer between the therapeutic agent and the water-soluble polymer, wherein the spacer is uncleavable.

8. The pharmaceutical composition of claim 2, wherein the targeting moiety directs the composition to a specific tissue.

9. The pharmaceutical composition of claim 2, wherein the targeting moiety directs the composition to bone or cartilage.

10. The pharmaceutical composition of claims 2, 8 or 9, wherein the targeting moiety is selected from the group consisting of bisphosphonates, quaternary ammonium groups, peptides, oligo-Asp, oligo-Glu, aminosalicylic acid, antibodies and fragments or derivatives thereof.

11. The pharmaceutical composition of claims 2, or 8-10, wherein the link between the targeting moiety and the water-soluble polymer is cleavable.

12. The pharmaceutical composition of claims 2, or 8-10, wherein the link between the targeting moiety and the water-soluble polymer is uncleavable.

13. The pharmaceutical composition of any one of claims 1-11, wherein the water-soluble polymer comprises N-(2-hydroxypropyl)methacrylamide.

14. The pharmaceutical composition of any one of claims 1-3 and 5-13, wherein the water-soluble polymer comprises one or more monomers selected from the group consisting of, N-(2-hydroxypropyl)methacrylamide, *N*-isopropylacrylamide, acrylamide, *N,N*-dimethylacrylamide, *N*-vinylpyrrolidone, vinyl acetate, 2-methacryloxyethyl glucoside, acrylic acid, methacrylic, vinyl phosphonic acid, styrene sulfonic acid, maleic acid, 2-methacrylloxyethyltrimethylammonium chloride, methacrylamidopropyltrimethylammonium chloride, methacryloylcholine methyl sulfate, *N*-methylolacrylamide, 2-hydroxy-3-methacryloxypropyltrimethyl ammonium chloride, 2-methacryloxyethyltrimethylammonium bromide, 2-vinyl-1-methylpyridinium bromide, 4-vinyl-1-methylpyridinium bromide, ethyleneimine, (*N*-

acetyl)ethyleneimine, (*N*-hydroxyethyl)ethyleneimine, allylamine and combinations thereof.

15. The pharmaceutical composition of any one of claims 1-14, wherein the therapeutic agent is selected from the group consisting of proteins, peptides, NSAIDs, glucocorticoids, methotrexate, sulfasalazine, chloriquine, gold, gold salt, copper, copper salt, penicillamine, D-penicillamine, cyclosporine, and mixtures thereof.

16. A method for the treatment of an inflammatory disease comprising: administering a composition to a subject thought to have an inflammatory disease, wherein the composition comprises a water-soluble polymer and an effective amount of a therapeutic agent linked to said water-soluble polymer.

17. The method according to claim 16, further comprising targeting the water-soluble polymer to a specific tissue.

18. The method according to of claims 16 or 17, wherein the inflammatory disease comprises arthritis.

19. The method according to claims 16, 17 or 18, wherein the water-soluble polymer is selected from the group consisting of a HPMA copolymer, polyethylene glycol, polyglutamic acid, polyaspartic acid, dextran, chitosan, cellulose, starch, gelatin, hyaluronic acid and derivatives thereof.

20. The method according to any of claims 16-19, conducting a biodistribution assay wherein the composition is labeled.

21. The method according to any of claims 16-20, further comprising cleaving the link between the therapeutic agent and the water-soluble polymer.

22. The method according to claim 17, wherein the targeting the water-soluble polymer to a specific tissue comprises targeting bone or cartilage.

23. The method according to claims 17 or 19, wherein targeting the water-soluble polymer to a specific tissue comprises using a targeting moiety selected from the group consisting of bisphosphonates, quaternary ammonium groups, peptides, oligo-Asp, oligo-Glu, aminosalicylic acid, antibodies and fragments or derivatives thereof.

24. The method according to claims 17, or 23, further comprising cleaving the link between the targeting moiety and the water-soluble polymer.

25. A method of administering an aqueous composition to a subject, said method comprising:

administering a composition of claim 1 in an aqueous solvent or diluent to a subject thought to have arthritis; and

allowing accumulation and targeting of the composition in arthrititic joint, thereby improving a treatment of arthritis.

26. The method according to claim 25, further comprising reducing a side effect of the therapeutic agent in tissues other than the arthritic joint.

27. The method according to claim 25, wherein the therapeutic agent is selected from the group consisting of a cycloxygenase-2 inhibitor, a glucocorticoid, a tumor necrosis factor blocker and an interleukin-1 receptor antagonist.

28. The method according to claim 25, wherein the water-soluble agent comprises a HPMA copolymer.

29. A composition for imaging and evaluation of an inflammatory disease comprising:

a water-soluble polymer and an effective amount of a medical imaging agent linked to said water-soluble polymer, wherein medical imaging agent is used in the imaging and evaluation of an inflammatory disease.

30. The composition of claim 29, further comprising an effective amount of a therapeutic agent (agents) linked to said water-soluble polymer.

31. The composition of claim 29 or 30, wherein the medical imaging agent is selected from the group consisting of at least one of a MRI, PET, CT and γ-scintigraphy agent.

32. The composition of claim 29, further comprising a targeting moiety linked to the water-soluble polymer.

33. The composition of claim 29, wherein the inflammatory disease comprises arthritis.

34. The composition of claims 29, 30, 31, 32 or 33, wherein the water-soluble polymer is selected from the group consisting of a HPMA copolymer, polyethylene glycol, polyglutamic acid, polyaspartic acid, dextran, chitosan, cellulose, starch, gelatin, hyaluronic acid and derivatives thereof.

35. The composition of any one of claims 29-34, further comprising a bio-assay label linked to the water-soluble polymer.

36. The composition of any one of claims 29-35, further comprising a spacer between the imaging agent and the water-soluble polymer, wherein the spacer is cleavable.

37. The composition of any one of claims 29-35, further comprising a spacer between the imaging agent and the water-soluble polymer, wherein the spacer is uncleavable.

38. The composition of claim 30, further comprising a spacer between the therapeutic agent and the water-soluble polymer, wherein the spacer is cleavable.

39. The composition of claim 30, further comprising a spacer between the therapeutic agent and the water-soluble polymer, wherein the spacer is uncleavable.

40. The composition of claim 29, wherein the targeting moiety directs the composition to a specific tissue.

41. The composition of claim 40, wherein the targeting moiety directs the composition to bone or cartilage.

42. The composition of claim 40, wherein the targeting moiety is selected from the group consisting of bisphosphonates, quaternary ammonium groups, peptides, oligo-Asp, oligo-Glu, aminosalicylic acid, antibodies and fragments or derivatives thereof.

43. The composition of claims 2, or 7-9, further comprising a targeting moiety linked to the water-soluble polymer.

44. The composition of claim 29, wherein the water-soluble polymer comprises N-(2-hydroxypropyl)methacrylamide.

45. The composition of claim 29, wherein the water-soluble polymer comprises one or more monomers selected from the group consisting of N-(2-hydroxypropyl)methacrylamide, *N*-isopropylacrylamide, acrylamide, *N,N*-dimethylacrylamide, *N*-vinylpyrrolidone, vinyl acetate, 2-methacryloxyethyl

glucoside, acrylic acid, methacrylic, vinyl phosphonic acid, styrene sulfonic acid, maleic acid, 2-methacrylloxyethyltrimethylammonium chloride, methacrylamidopropyltrimethylammonium chloride, methacryloylcholine methyl sulfate, *N*-methylolacrylamide, 2-hydroxy-3-methacryloxypropyltrimethyl ammonium chloride, 2-methacryloxyethyltrimethylammonium bromide, 2-vinyl-1-methylpyridinium bromide, 4-vinyl-1-methylpyridinium bromide, ethyleneimine, (*N*-acetyl)ethyleneimine, (*N*-hydroxyethyl)ethyleneimine, allylamine and combinations thereof.

46. The composition of claim 30, wherein the therapeutic agent is selected from the group consisting of proteins, peptides, NSAIDs, glucocorticoids, methotrexate, sulfasalazine, chloriquine, gold, gold salt, copper, copper salt, penicillamine, D-penicillamine, cyclosporine, and mixtures thereof.

47. A method for imaging and evaluation of an inflammatory disease, the method comprising:

administering the imaging agent of claim 29; and

imaging an inflammatory disease patient or animal model before and after the administration of the imaging agent with MRI, PET, CT or γ-scintigraphy equipment.

48. The method according to claim 47, wherein the water-soluble polymer is selected from the group consisting of a HPMA copolymer, polyethylene glycol, polyglutamic acid, polyaspartic acid, dextran, chitosan, cellulose, starch, gelatin, hyaluronic acid and derivatives thereof.

49. The method according to claims 47 or 48, further comprising conducting a biodistribution assay.

50. The method according to claim 47, 48 or 49, further comprising targeting the water-soluble polymer to a specific tissue.

51. The method according to claim 50, wherein targeting of the compound is directed to bone or cartilage.

52. The method according to claims 50 or 51, wherein targeting the compound to a specific tissue comprises using a targeting moiety selected from the group consisting of bisphosphonates, quaternary ammonium groups, peptides, oligo-Asp, oligo-Glu, aminosalicylic acid, antibodies and fragments or derivatives thereof.

53. The method according to any one of claims 50-52, further comprising cleaving a link between the targeting moiety and the water-soluble polymer.

54. The method according to claim 50, wherein imaging an inflammatory disease patient or animal model enhanced with the compound comprises imaging an arthritic joint.

55. The method according to claim 47, wherein the medical imaging agent is selected from the group consisting of at least one of a MRI, PET, CT, γ-scintigraphy agent and combinations thereof.

56. The method according to claim 47, wherein the water-soluble agent comprises a HPMA copolymer.

57. The pharmaceutical composition of claim 1, wherein the therapeutic agent comprises a plurality of distinct therapeutic agents.

58. The pharmaceutical composition of claims 2, 8 or 9, wherein the targeting moiety comprises a plurality of distinct targeting moieties.

59. The pharmaceutical composition of claim 58, wherein the plurality of distinct targeting moieties target a plurality of tissues.

60. The pharmaceutical composition of claim 5, wherein the bio-assay label comprises a plurality of distinct bio-assay labels.

61. The pharmaceutical composition of claim 6 or claim 7, wherein the spacer comprises a plurality of chemically distinct spacers.

62. The composition of claim 31, wherein the imaging agent comprises a plurality of distinct imaging agents.

63. The method according to claim 55, wherein the imaging agent comprises at least two imaging agents, wherein each of the two imaging agents is used in a different imaging technique.

ABSTRACT

This invention relates to biotechnology, more particularly, to water-soluble polymeric delivery systems for the imaging, evaluation and/or treatment of arthritis and other inflammatory diseases. Using modern MR imaging techniques, the specific accumulation of macromolecules in arthritic joints in adjuvant-induced arthritis in rats is demonstrated. The strong correlation between the uptake and retention of the MR contrast agent labeled polymer with histopathological features of inflammation and local tissue damage demonstrates the practical applications of the macromolecular delivery system of the invention. In addition, treatment data confirmed the advantages of the polymeric delivery system in the treatment of experimental rheumatoid arthritis in an animal model.

FIG. 1.

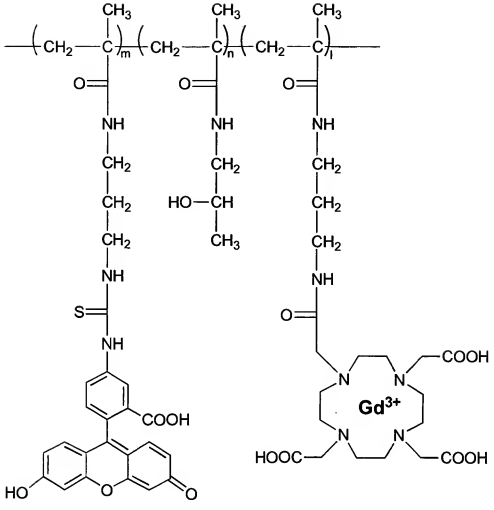
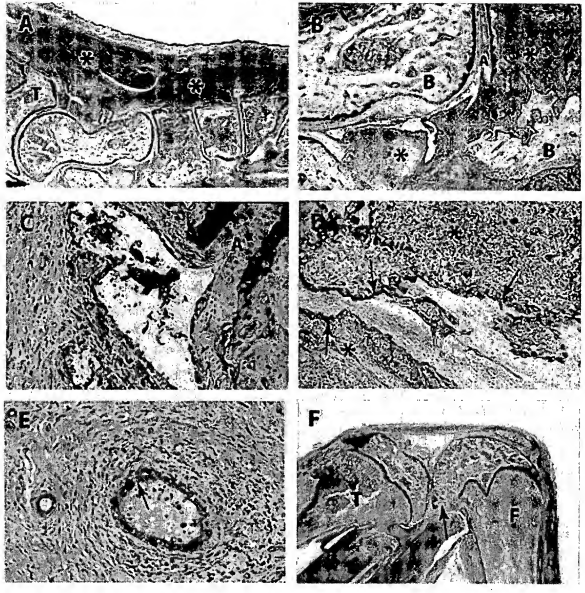


FIG. 2.



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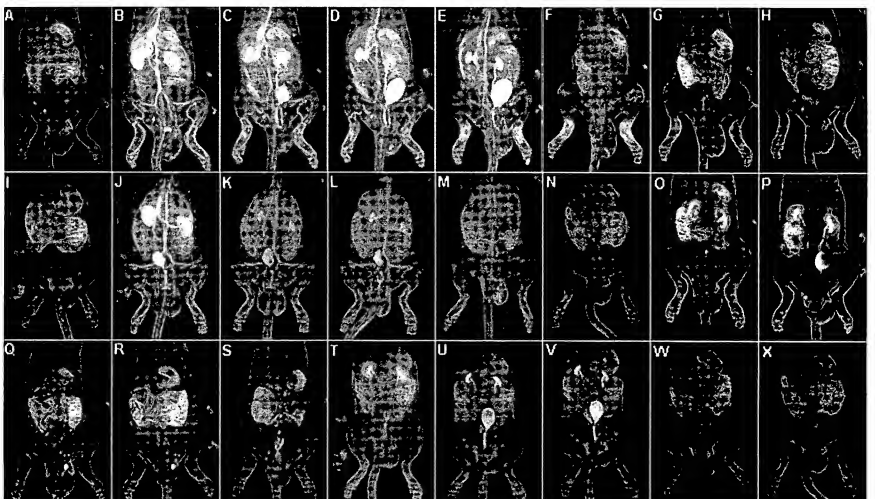

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FIG. 4.

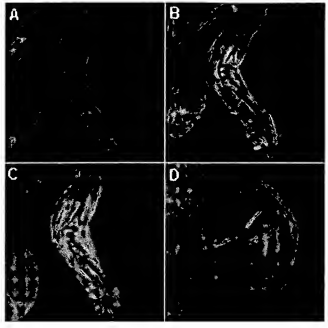

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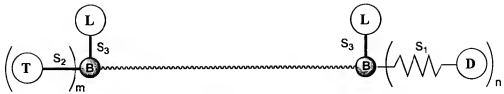
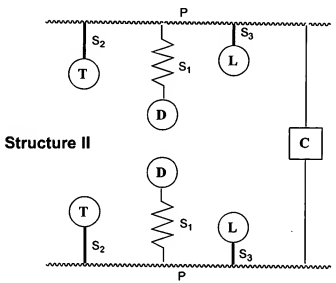
FIG. 5.



Structure I



Structure II

T Targeting moieties covalently bonded to the polymer terminus or backbone via spacer S_2

D Anti-inflammatory drugs or non-invasive imaging agentss or mixture of both,bonded to the polymer via spacer S_1

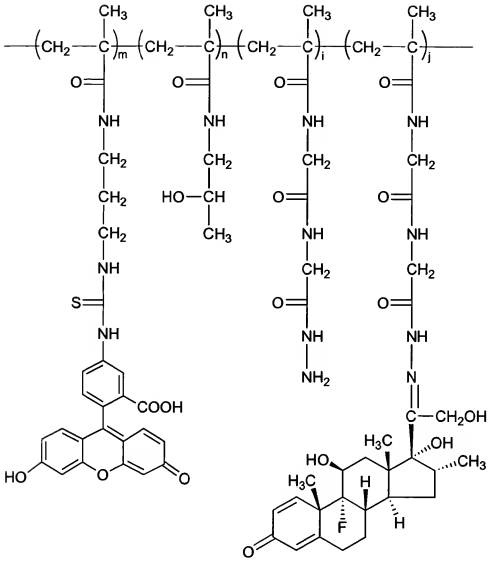
L Optional bio-assay label covalently bonded to the polymer backbone via non-degradable spacer S_3

B Branching structure.

C Optional biodegradable cross-linkage in the delivery system

Inert polymeric backbone of the delivery system, P

FIG. 6.


CH3
CH2
C
m
n
i
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O
NH
CH2
HO—CH
CH3
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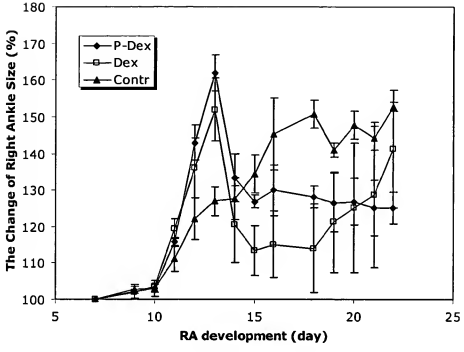
C—CH2OH
H3C
OH
CH3
H
F

FIG. 7.


P-Dex
Dex
Contr
The Change of Right Ankle Size (%)
RA development (day)
180
170
160
150
140
130
120
110
100
5
10
15
20
25